Neurochemical alterations after exposure to malathion and neuroprotective potential of cerebrolysin as therapeutic agent against malathion toxicity in rats

Abd El-Hamid Mohamed Elwy[1], and Ghada Tabl[2]

[1] Ph.D., Assistant Professor, Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Tanta University, Tanta City-Gharbia Governorate- Egypt
[2] Ph.D., Zoology Department, Faculty of Science, Tanta University, Tanta City-Gharbia Governorate- Egypt
Email. Elwyabdelhamid@gmail.com

Abstract: Malathion (MAL) is a neurotoxin organophosphate (OP) widely used as insecticide. Although, malathion is an organophosphate pesticide agent used in many fields of most countries, it leads to the toxicity at different degrees. Malathion has widespread use especially in developing countries, so it may increase the mortality rate which is a pertinent health problem. However, although, malathion is known as a pesticide, it is still used in medicine. In fact humans and other animals are exposed to low doses of malathion (MAL). Cerebrolysin (CBL) is a drug that possess neurotrophic factor like- activity and neuroprotective properties, as well as it has small brain-derived peptides analogous to those produced endogenously, which have the ability to cross through the blood-brain barrier. Aim: The present investigation has been conducted to verify the effect of cerebrolysin as a neuroprotective drug against malathion neurotoxicity in a mammalian experimental model. Main method: Wistar male rats were used in the present study with initial body weight 180 g, ranging from 180–220 g. All rats were individually housed in plastic cages with a photo cycle of a 12-hour/12-hour light/dark cycle. The Wistar male rats, were randomly divided into four groups (8-rats/group). 1- control group was administered normal saline orally via gastric tube, 2- Cerebrolysin treated group was daily administered intraperitoneally with 2.5 ml/kg 3- malathion treated group was administered orally 5 mg/ kg, daily for 28 days,. 4- malathion + cerebrolysin treated group, received cerebrolysin 2.5 ml/kg at the same time after malathion administration for 28 days. At the end of each experiment. Rats were sacrificed quickly with the least disturbance by fast decapitation, which may occur within a few minutes. Results. There was a significant decrease in the mean acetylcholinesterase (AChE) concentrations. in malathion treated group. Whereas, data showed a significant increase in both mean concentrations of MDA and TNFα. The excitatory amino acids; glutamic acid showed a significant increase, while the inhibitory amino acids; γ-amino- butyric acid (GABA) showed a significant decrease in their levels, as well as there was a significant decrease after oral administration of malathion in the dopamine levels. The alterations in these parameters accompanied malathion toxicity in rats showed improvement in their levels after therapeutic influence of cerebrolysin administration. This could suggest to be due to neurotrophic factor like- activity and neuroprotective properties of cerebrolysin. Conclusion: The results of the present study revealed that malathion toxicity caused oxidative stress to the brain cells (brain damage) indicated by an increases in both MDA and TNFα which may increase the mortality rate and considered as a pertinent health problem. As well as, after exposure to malathion, some general toxic observations were detected to some animals. Some rats showed noticeable behavioral neurotoxicological changes as excitation and disturbances of the locomotors activity. In summary, cerebolysin protected the exposed animals against neurotoxicity induced by malathion through the inhibition of oxidative stress and inflammatory cytokine; TNFα. As well as, through the improvement in both dopamine levels and in mean acetylcholinesterase (AChE) concentration.
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1. Introduction

Malathion is a neurotoxin organophosphate (OP) widely used as insecticide. It was introduced in 1950 (1) as one of the earliest organophosphate insecticides and it remains in use up tell now. Malathion used in agriculture to control pests, in residential landscaping and in public health program (2). Beside its application to plants, malathion is considered as a personal hygiene products (3), also it is used in public health pest control programs as mosquito and lice (4). As well as, it is used as an ectoparasiticide in public health programs that is utilized against human head and body lice (5). Also, malathion is confirmed by the US Food Drug Administration for the treatment of pediculosis, which causing risk to human health (6; 7). Although, malathion is an organophosphate pesticide

agent used in many fields of most countries, it leads to the toxicity at different degrees. Even though, malathion is known as a pesticide, it is still used in medicine. Thereby, organophosphates including malathion remain a serious health hazard (8).

Malathion has a severe toxic effect on neural regulation by reducing acetyl cholinesterase (AChE) in the nervous tissue with a resultant increase in the levels of acetylcholine, causing several cholinergic symptoms (9). Malathion is efficient against a wide assortment of insects as: ants, fleas, fruit flies, wasps, ticks, mites, mosquitoes, moths, and spiders. Malathion can be applied by aircrafts, duster, fogger, irrigation, sprayer or spreader. It is available in many different formulations, such a ready-to-use liquid, dust, and pressurized liquid (1). The absorption or ingestion of malathion into the human body results in its metabolism to malaoxon, which is more toxic and powerful than the malathion itself and has been estimated to be 61 times more toxic than malathion (10). Moreover, it is well known that malathion induces its toxicity through its metabolite, malaxone which is more harmful than malathion (11). It is cleared from the body, in three to five days (12). Environmental problems of pollution of rivers and streams are caused by chemical contaminants from industrial areas to aquatic organisms causing toxicity risk to those organisms, as a result of the pollutants transportation and accumulations in their edible parts (13;14). Insecticides represent a variety of chemicals, most of which target the nervous system and disrupt neural regulation (15).

Cerebrolysin is a mixture of peptides purified from pig brains (16;17). In fact, humans and other animals are exposed to low doses of malathion insecticides. Since, malathion has the prevalent (widespread) use especially in developing countries, it may increase the mortality rate which is a pertinent health problem due to toxic effects from malathion exposure (18). Thereby, the present investigation was undertaken to verify the protective effect of the cerebrolysin which has Neurotrophic activities as a drug against malathion induced neurotoxicity in rats.

2. Material and Methods

Material:

Animals

Male Wistar rats were used in the present study with initial body weight 180 gm, ranging from 180–220 g. All rats were individually housed in metabolic plastic cages under the same environmental conditions in a well ventilated animal controlled room temperature ranging from 22°C–27°C with a photo cycle of a 12-hour/12-hour light/dark cycle. The experimental rats were fed *ad libitum* with a standard diet and allowed free access of water and maintained on a 12-h light/dark cycle. The rats were randomly distributed into specified experimental groups. All the experimentations used in this study were carried out in an ethical manner following guide lines for scientific research. As well as, all the experiments were carried out at fixed time of the day (10-12 O'clock) to minimize the daily fluctuations in brain monoamines.

Drugs and chemicals

Cerebrolysin (CBL) was purchased from EVER Neuropharma GmbH A-4866 Unterach, Austria.

Malathion was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

All other reagents were analytical grade reagents.

Rout of administration

Malathion 5 mg/kg was administered orally and daily for 28 days (19).

Cerebrolysin was administered intraperitoneally and daily (2.5 ml/kg, each ml contains 215.2 mg CBL) at the same time after malathion administration for 28 days (20).

Experimental design

Male Wistar rats were randomly divided into four groups (8-rats/group).

1-First group served as control group and received normal saline;

2- Second group treated with cerebrolysin 2.5 mg/kg.

3- Third group treated with malathion 5 mg/ kg.

4-Fourth group treated with both malathion 5 mg/ kg + cerebrolysin 2.5 mg/kg.

At the end of each experiment, rats were sacrificed quickly with least disturbance by fast decapitation to avoid any substantial changes in brain amines, which may occur within a few minutes.

Methods and Techniques:

Determination of acetylcholinesterase (AChE) activity

AChE activity was measured by the method of Ellman et al. (21), using acetylthiocholine iodide as a substrate in homogenates of cerebral cortex centrifuged at 2300 r.p.m. for 15 min. The rate of hydrolysis of acetylthiocholine iodide was measured at 412 nm through the release of the thiol compound which when reacted with DTNB produced the color-forming compound thionitrobenzoic acid.

3.2-Measurement of MDA in the cerebral cortex tissue

The levels of the MDA were measured according to the method of Fernandez et al. (22). This method is based on the spectrophotometric measurement of the color produced by reaction of MDA to thiobarbituric acid (TBA). Tissue MDA levels were expressed as nmol/g tissue.

Detection of TNF-α in tissue

The frozen tissue (cerebral cortex) was homogenized with a homogenizer in 1 ml of buffer containing 0.5 ml lysis buffer Tris– NaCl, pH 7.6. and centrifuged at 10,000g for 10 min at 4 °C. Supernatant was separated and protein content was immediately measured using Bio-Rad protein assay kit. TNF-α level in the supernatants were determined using an ELISA kit (Invitrogen, USA) specific for rat TNF-α. The measurement of TNF-α was carried out according to the protocol booklet of the ELISA kit based on the manufacturer's instructions (23).

Neurotransmitters assay:

High performance liquid chromatography (HPLC) determination of the free amino acid contents in the brain cortex extracts

Brain free amino acids; the excitatory amino acid; glutamic acid, as well as inhibitory amino acid; GABA (γ-amino- butyric acid), were detected by high performance liquid chromatography (HPLC) using the pre column PTC derivatization technique according to the method of Hassel et al. (24).

3.5-Determination of dopamine level in cerebral cortex

The dopamine level was quantified in the supernatant of cerebral cortex tissue using the method reported by Calderon et al. (25). Tissue dopamine level was expressed as μmol /g.

Statistical analysis

The data obtained were statistically analyzed using F-test (ANOVA) by SPSS Version 9. Effects with a probability of $p < 0.05$ were considered to be significant.

3. Results and discussion

Neurotransmitters are fundamental to all central nervous system. The mechanism of action of neurotoxic drugs is through one of this neurotransmitter's system. The most common known neurotransmitters are acetylcholine, amino acids including some inhibitory & excitatory amino acids and catecholamines (26). The present investigation focus on the response of the brain cells towards the malathion as a neurotoxic drug (1) on acetylcholinesterase, individual free amino acids, include some excitatory (glutamic acid) and inhibitory (gamma – amino butyric acid (GABA)) amino acids, dopamine, malondialdhyde (MDA) and TNFα in brain tissues of Wister rats at selected time period 28 days of exposure, as well as the neuroprotective influence of cerebrolysin as a neurotrophic factor

Acetylcholinesterase activity in malathion toxicated rats and the effect of cerebrolysin as a neuroprotective factor.

Acetylcholinesterase activity is essential for the healthy function of the brain tissues. The differences in AChE activity are known to be accompanied by clear signs of neurobehavioral toxicity. Therefore, this parameter could be used as a neurotoxicity index in animals and humans (27).

The collective data of acetylcholinesterase Activities in the tissues of cerebral cortex of experimental rats affected by continuous administration of malathion 5mg/Kg for 28 days time period, illustrated in table (1), the present results showed significant decrease in brain acetylcholinesterase (AChE) activity. The results of the present study come in agreement with several investigations that showed subacute malathion administration caused a significant reduction in AChE activity (28). The data of the present investigation suggest the decrease in brain levels of AChE ($p < 0.001$), could explain in part the noticeable behavioral neurotoxicologicaL disturbances of the locomotors activity observed in the experimental model during the work. On the other hand, there was statistically significant changes in AChE activities between malathione group and Cerebrolysin and malathion co-administered group, this could suggest an evidence that cerebrolysin might exert its inhibitory effect with 15.0% on brain acetylcholinesterase (AChE) activities through its action as a neurotrophic factor against malathion induced neurotoxicity in rats. This comes in accordance with Selmi et al. (29) who reported that organophosphates, including malathion, have been indicated to exert their primary pharmacological and toxicological effects through the inhibition of acetylcholinesterase (AChE) activity.

Table (1): Effect of cerebrolysin on acetylcholinesterase (AChE) in malathion toxicated rats

	Control (n=8)	CBL (n=8)	M (n=8)	CBL + M (n=8)	F	p
AchE nmol/min/g)	13.3 ± 0.2	13.2 ± 0.2	10 ab± 0.3	11.5 abc± 0.3	274.64	<0.001*
% of Change			↑15.0			

F, p: F and p values for ANOVA test, Pair wise comparison bet. Each 2 groups was done using Post Hoc Test (Tukey)

*: Statistically significant at $p \leq 0.05$ a: Significant with control b: Significant with CBL

c: Significant with M CBL: Cerebrolysin M: Malathion CBL + M: Cerebrolysin + Malathion

Malondialdehyde (MDA) alterations in malathion intoxicated rats and the potential therapeutic effect of cerebrolysin as a neurotrophic agent.

In view of the fact that, the high levels of polyunsaturated fatty acids, high utilization of oxygen and the plenty of redox-active transmission metal ions makes the brain more susceptible to oxidative damage. Moreover, as oxidative stress is a pathophysiological mechanism where there is an imbalance between concentrations of reactive oxygen species (ROS) and antioxidants. However, excessive ROS accumulation will lead to cellular injury and damage to lipid membranes due to lipid peroxidation which represented by the increase in the MDA levels. Moreover, the lipophilic nature of malathion facilitated its interaction with cell membrane and leads to perturbations of the phospholipid bilayer structure (30) Furthermore, in addition to cholinesterase inhibition in malathion intoxicated rats, malathion can induce oxidative stress in different tissues including the brain in acute and chronic exposures. Therefore, excessive ROS must be instantly discarded from the cells because of their potential harmful effect. (31) As, the cerebrolysin (CBL) possess neurotrophic factor like- activity and neuroprotective properties (20; 32) as well as due to, its small brain derived peptides analogous to those produced endogenously, which have the ability to cross through the blood-brain barrier (33). Also, it has been successfully investigated in animal models, in which it was proved to decrease oxidative stress (34). Moreover, the neuroprotective properties of cerebrolysin (CBL) are correlated with its effectiveness in the treatment of neurodegenerative disorder (32). Furthermore, due to the neurotrophic effects of CBL that interferes with excitotoxicity free radical formation which cause the peroxidation assessed by the increased MDA levels to the cell membrane and inflammatory responses (35; 36; 37). Therefore, it was interesting to investigate the potential therapeutic influence of cerebrolysin (CBL) on some chemical alterations accompanied malathion toxicity in rats as an experimental model.

Data of the present investigation (Table 2) showed that, the daily administration of malathion at dose level 5mg/Kg produced significant increase in lipid peroxidation ($p < 0.001$), as assessed by increased malondialdehyde (MDA) levels. On the other hand, cerbrolysin at dose level 2.5 mg/Kg resulted in reduce MDA level in the cerebral cortex of the experimental rats. The data of the present investigation provide evidence that the co-administration of cerebrolysin with malathion had reduced the MDA concentration in the cerebral cortex of rats (Table2). Also, these data suggest that cerebrolysin may exert its neuroprotictive effect through the enhancement of the antioxidant activity which acts to keep intracellular redox equilibrium and thus maintains the cell against oxidative injury and as a direct free-radical scavenger. Another explanation, the significant increase in MDA levels suggests the enhanced lipid peroxidation which leading to tissue damage, due to failure of antioxidant defense mechanism system. This assumption is in the same way with Amresh et al. (38). Another support to this concept comes from the fact that the high levels of polyunsaturated fatty acids, high utilization of oxygen and the plenty of redox-active transmission metal ions makes the brain more sensitive to oxidative damage (39) Furthermore, the antioxidant and neurotrophic activities of CBL have been reported in other models. Moreover, Abdel-Salam et al. (2; 40) demonstrated that CBL exerts inhibitory effect on the elevation of brain MDA. In the same way, data of the present investigation are in accordance with Patočková et al. (41) who reported that a single injection of CBL significantly reduce brain lipid peroxidation in a mice-model and hence lower the MAD levels.

Table (2): Effect of cerebrolysin on malondialdehyde (MDA) in malathion toxicated rats

	Control (n=8)	CBL (n=8)	M (n=8)	CBL + M (n=8)	F	p
MDA (µmol/ g tissue)	8.4± 0.3	8.2± 0.2	11.7ab± 0.4	9.1abc± 0.2	260.80*	<0.001*
% of Change			↓22.22			

F, p: F and p values for ANOVA test, Pair wise comparison bet. Each 2 groups was done using Post Hoc Test (Tukey)

*: Statistically significant at $p \leq 0.05$ a: Significant with control b: Significant with CBL

c: Significant with M CBL: Cerebrolysin M: Malathion CBL + M: Cerebrolysin + Malathion

Cytokines production, (TNF-α) in malathion-intoxicated rats and the possible effects of cerebrolysin as a neurotrophic agent against malathion toxicity

The results of the present investigation (Table 3) showed signs of damage in the brain of intoxicated rats after malathion administration which indicated by increasing the cytokines production (TNF-α) and malondialdehyde (MDA) levels which give another support to the brain damage of intoxicated rats. On contrary, rats treated with cerebrolysin exhibited significantly lower TNF-α. This comes in accordance

with Lin et al. (42) who reported that TNF-α is one of the molecules that involved in the various stages of inflammation, hence CBL succeeded in restoring and normalized the increased TNF-α levels recorded in malathion intoxicated rats by percentage inhibition 68.72.

Amino acids, glutamic acid and γ-amino- butyric acid (GABA) in malathion intoxicated rats and the possible influence of cerebrolysin as a neurotrophic agent against malathion toxicity.

The important mechanisms for excitatory and inhibitory neurotransmission are the neurotransmitters synthesis and subsequent uptake and storage in synaptic vesicles in adequate amount (43). The neutral amino acids such as γ-amino- butyric acid (GABA) and the acidic amino acids, such as glutamate are present in high concentrations in the central nervous system (CNS) and are highly potent modifiers of the neuronal excitability. Moreover, glutamate, serves as an energy substrate, a building unit of proteins and an excitatory neurotransmitter (43). Furthermore, Rudolph et al. (44) reported that glutamate is the major and most common neurotransmitter in the CNS.

Table (3): Effect of cerebrolysin on tumor necrosis factor (TNF-α) in malathion toxicated rats

	Control (n=8)	CBL (n=8)	M (n=8)	CBL + M (n=8)	F	p
TNF-α (pg/mg protein)	18.8± 0.4	18.6± 0.3	81.2 ab±1.5	25.4abc± 0.8	9020.19*	<0.001*
% of Change			↓68.72			

F, p: F and p values for ANOVA test, Pair wise comparison bet. Each 2 groups was done using Post Hoc Test (Tukey) *: Statistically significant at p ≤ 0.05 a: Significant with control b: Significant with CBL
c: Significant with M CBL: Cerebrolysin M: Malathion CBL + M: Cerebrolysin + Malathion

The present results demonstrated that the daily oral administration of malathion produced a significant increase in the mean of glutamic acid concentrations ($p < 0.001$) and reduce in the mean of GABA concentrations ($p < 0.001$) (Table4). The significant increase in the glutamic acid concentrations may be attributed to their release from the degenerated nerve cells and rupture of synaptic bolus produced by repeated administration of malathion. This assumption is strongly supported by the increase in the MDA level, beside the increased cytokines production, (TNF-α) which are usually considered characteristics of cells and hence brain damage and probably contributes to the rat cerebral dysfunction and confirmed the degenerative effect of repeated malathion administrations. Additional, support to the concept of the relationship between nerve cell injury and increased brain levels of glutamic excitatory amino acids comes from the work of Kong et al. (45) who observed the amount of excitatory amino acids in hippocampus was raised, especially that of glutamic acid after administration of excessive zinc in young rats. Beside these reports, the author observed that the neurons and the neuroglial cells showed degeneration and rupture of synaptic bolus.

Table (4): Effect of cerebrolysin on GABA and glutamate in malathion toxicated rats

	Control (n=8)	CBL (n=8)	M (n=8)	CBL + M (n=8)	F	p
GABA µmol/g tissue	2.4± 0.1	2.4± 0.2	1.1ab± 0.1	1.9 abc± 0.2	137.97*	<0.001*
% of Change			↑72.73			
Glutamate (µmol/g tissue)	9.8± 0.2	9.8± 0.3	12.6 ab± 0.4	10.4 abc± 0.3	123.85*	<0.001*
% of Change			↓17.46			

F, p: F and p values for ANOVA test, Pair wise comparison bet. Each 2 groups was done using Post Hoc Test (Tukey) *: Statistically significant at p ≤ 0.05 a: Significant with control b: Significant with CBL
c: Significant with M CBL: Cerebrolysin M: Malathion CBL + M: Cerebrolysin + Malathion

Furthermore, the significant increase in glutamic excitatory amino acid might explain and support the previously observed convulsions and the abnormalities in the locomotor organs in rats administered daily for 28 days by malathion. Another possible reason for the increase in glutamic acids concentration detected after malathion administration could be their accumulation at the synapses due to the malathion administration -induced inhibition of neuronal glutamate receptors on the postsynaptic level. As well as, the results indicated the inhibitory effect of malathion administration through the significant reduction in the inhibitory amino acid GABA concentration (Table4) The reduction in GABA concentrations detected in the cerebral cortex extracts may be through decreased its neuronal

synthesis and is affected by lack of synaptic vesicles causes down- regulation of neuronal GABA. This proposal is in agreement with Bogen et al. (46). However, the present results demonstrated that the use of cerebrolysin, in view of its properties that it possess neurotrophic factor like- activity and neuroprotective properties (20; 32), the current results showed that the cerebrolysin has been successfully improved the levels of the excitatory and the inhibitory amino acids according its properties by percentage increase in GABA level 72.73 and percentage decreased in glutamate level with 17.46.

Dopamin in malathion-intoxicated rats and the possible effects of cerebrolysin as a neurotrophic agent against malathion toxicity.

In the present study the results (Table 5) showed a statistically significant decrease in the dopamine levels ($p < 0.001$), after oral administration of malathion, this could be attributed to the degeneration of dopaminergic neurons which detected through the increased lipid peroxidation of the membrane which assessed by increased MDA. Where as, coadministration of cererolysin with malathion resulted in increased dopamine levels in the cortex region though, it could be assumed that cerebrolysin is a dopamine agonist. Furthermore, the present results in accordance with Guzmán et al. (47) who reported that cerebrolysin has a dopaminergic effect on neurodegeneration of rat with oxidative stress induced by 3-nitropropionic.

Table (5): Effect of cerebrolysin on dopamine in malathion toxicated rats

	Control (n=8)	CBL (n=8)	M (n=8)	CBL + M (n=8)	F	p
Dopamine (µmol/g)	3.4± 0.1	3.4± 0.2	2.4ab± 0.1	2.8abc± 0.3	78.16*	<0.001*
% of Change			↑16.67			

F, p: F and p values for ANOVA test, Pair wise comparison bet. Each 2 groups was done using Post Hoc Test (Tukey) *: Statistically significant at $p \leq 0.05$ a: Significant with control b: Significant with CBL
c: Significant with M CBL: Cerebrolysin M: Malathion CBL + M: Cerebrolysin + Malathion

Disclosure

The authors declare that there is no conflict of interest that would prejudice the impartiality of the reported research.

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